Lab sheet #7

-Agarose Gel Electrophoresis-

-Objectives:

- To separate and calculate the molecular size of DNA fragment by comparing the separated bands with known standard molecular weight marker.

-Method:

1. Measure the desired grams of agarose to make 0.5% agarose gel.
2. Heat the solution to boiling in the microwave to dissolve the agarose to produce a homogeneous mixture.
3. Add 4 µl of ethidium bromide CAREFULLY to the dissolved agarose and mix.
4. Get a gel plate and a comb. Put the two dams into the slots on each side of the gel plate. Make sure that they fit tightly. Pour the melted agarose onto the gel plate in the electrophoresis tray.
5. Let the gel cool to room temperature.
6. Place the gel in the electrophoresis chamber.
7. Pour enough electrophoresis buffer (1X TBE) to cover the gel to prevent overheating of the gel.
8. Carefully remove the comb.
9. Prepare the DNA sample by mixing around 300 ng of DNA sample with 3-4 µl of loading dye.
10. Add 3 µl DNA ladder into the first well by using a micropipette.
11. Carefully place the prepared samples into adjacent wells.
12. Electrophorese the samples at 95 V for 45 minutes. (Check the gel while it is running).
13. Carefully remove the gel, place it onto the UV light box and take a picture for the gel.

-Results:

- Picture of the gel.

Related questions:

1. To which electrode do the nucleic acids migrate? Why?

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2. What is the relation between the nucleic acid fragment and migration?

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3. For what purpose is the ladder used?

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